



SEQUENCE LISTING

<110> Short, Jay M.
Kretz, Keith A.
Gray, Kevin A.
Barton, Nelson Robert
Garrett, James B.
O' Donoghue, Eileen
Mathur, Eric J.

<120> RECOMBINANT BACTERIAL PHYTASES AND USES
THEREOF

<130> 09010-029006

<140> US 09/866,379

<141> 2001-05-24

<150> US 09/580,515

<151> 2000-05-25

<150> US 09/318,528

<151> 1999-05-25

<150> US 09/291,931

<151> 1999-04-13

<150> US 09/259,214

<151> 1999-03-01

<150> US 08/910,798

<151> 1997-08-13

<160> 10

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<211> 1323

<212> DNA

<213> Escherichia coli

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<221> CDS

<222> (1)...(1320)

<221> misc_feature

<222> 216

<223> n = A,T,C or G

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1 5 10 15

ccg caa tct gca ttc gct cag agt gag ccg gag ctg aag ctg gaa agt 96

Pro	Gln	Ser	Ala	Phe	Ala	Gln	Ser	Glu	Pro	Glu	Leu	Lys	Leu	Glu	Ser	
			20					25					30			
gtg	gtg	att	gtc	agt	cgt	cat	ggg	gtg	cgt	gct	cca	acc	aag	gcc	acg	144
Val	Val	Ile	Val	Ser	Arg	His	Gly	Val	Arg	Ala	Pro	Thr	Lys	Ala	Thr	
		35					40					45				
caa	ctg	atg	cag	gat	gtc	acc	cca	gac	gca	tgg	cca	acc	tgg	ccg	gta	192
Gln	Leu	Met	Gln	Asp	Val	Thr	Pro	Asp	Ala	Trp	Pro	Thr	Trp	Pro	Val	
	50					55					60					
aaa	ctg	ggg	tgg	ctg	aca	ccg	cg	ggg	ggg	gag	cta	atc	gcc	tat	ctc	240
Lys	Leu	Gly	Trp	Leu	Thr	Pro	Arg	Gly	Gly	Glu	Leu	Ile	Ala	Tyr	Leu	
	65				70				75					80		
gga	cat	tac	caa	cgc	cag	cgt	ctg	gta	gcc	gac	gga	ttg	ctg	gcg	aaa	288
Gly	His	Tyr	Gln	Arg	Gln	Arg	Leu	Val	Ala	Asp	Gly	Leu	Leu	Ala	Lys	
			85					90						95		
aag	ggc	tgc	ccg	cag	tct	ggg	cag	gtc	gcg	att	att	gct	gat	gtc	gac	336
Lys	Gly	Cys	Pro	Gln	Ser	Gly	Gln	Val	Ala	Ile	Ile	Ala	Asp	Val	Asp	
			100					105					110			
gag	cgt	acc	cgt	aaa	aca	ggc	gaa	gcc	ttc	gcc	gcc	ggg	ctg	gca	cct	384
Glu	Arg	Thr	Arg	Lys	Thr	Gly	Glu	Ala	Phe	Ala	Ala	Gly	Leu	Ala	Pro	
		115					120					125				
gac	tgt	gca	ata	acc	gta	cat	acc	cag	gca	gat	acg	tcc	agt	ccc	gat	432
Asp	Cys	Ala	Ile	Thr	Val	His	Thr	Gln	Ala	Asp	Thr	Ser	Ser	Pro	Asp	
	130					135					140					
ccg	tta	ttt	aat	cct	cta	aaa	act	ggc	gtt	tgc	caa	ctg	gat	aac	gcg	480
Pro	Leu	Phe	Asn	Pro	Leu	Lys	Thr	Gly	Val	Cys	Gln	Leu	Asp	Asn	Ala	
	145				150					155					160	
aac	gtg	act	gac	gcg	atc	ctc	agc	agg	gca	gga	ggg	tca	att	gct	gac	528
Asn	Val	Thr	Asp	Ala	Ile	Leu	Ser	Arg	Ala	Gly	Gly	Ser	Ile	Ala	Asp	
				165					170					175		
ttt	acc	ggg	cat	cgg	caa	acg	gcg	ttt	cgc	gaa	ctg	gaa	cgg	gtg	ctt	576
Phe	Thr	Gly	His	Arg	Gln	Thr	Ala	Phe	Arg	Glu	Leu	Glu	Arg	Val	Leu	
			180					185					190			
aat	ttt	ccg	caa	tca	aac	ttg	tgc	ctt	aaa	cgt	gag	aaa	cag	gac	gaa	624
Asn	Phe	Pro	Gln	Ser	Asn	Leu	Cys	Leu	Lys	Arg	Glu	Lys	Gln	Asp	Glu	
		195				200						205				
agc	tgt	tca	tta	acg	cag	gca	tta	cca	tcg	gaa	ctc	aag	gtg	agc	gcc	672
Ser	Cys	Ser	Leu	Thr	Gln	Ala	Leu	Pro	Ser	Glu	Leu	Lys	Val	Ser	Ala	
	210					215					220					
gac	aat	gtc	tca	tta	acc	ggg	gcg	gta	agc	ctc	gca	tca	atg	ctg	acg	720
Asp	Asn	Val	Ser	Leu	Thr	Gly	Ala	Val	Ser	Leu	Ala	Ser	Met	Leu	Thr	
	225				230				235					240		
gag	ata	ttt	ctc	ctg	caa	caa	gca	cag	gga	atg	ccg	gag	ccg	ggg	tgg	768
Glu	Ile	Phe	Leu	Leu	Gln	Gln	Ala	Gln	Gly	Met	Pro	Glu	Pro	Gly	Trp	

245	250	255	
gga agg atc acc gat tca cac cag tgg aac acc ttg cta agt ttg cat			816
Gly Arg Ile Thr Asp Ser His Gln Trp Asn Thr Leu Leu Ser Leu His			
260	265	270	
aac gcg caa ttt tat ttg cta caa cgc acg cca gag gtt gcc cgc agc			864
Asn Ala Gln Phe Tyr Leu Leu Gln Arg Thr Pro Glu Val Ala Arg Ser			
275	280	285	
cgc gcc acc ccg tta ttg gat ttg atc atg gca gcg ttg acg ccc cat			912
Arg Ala Thr Pro Leu Leu Asp Leu Ile Met Ala Ala Leu Thr Pro His			
290	295	300	
cca ccg caa aaa cag gcg tat ggt gtg aca tta ccc act tca gta ctg			960
Pro Pro Gln Lys Gln Ala Tyr Gly Val Thr Leu Pro Thr Ser Val Leu			
305	310	315	320
ttt att gcc gga cac gat act aat ctg gca aat ctc ggc ggc gca ctg			1008
Phe Ile Ala Gly His Asp Thr Asn Leu Ala Asn Leu Gly Gly Ala Leu			
325	330	335	
gag ctc aac tgg acg ctt ccc ggt cag ccg gat aac acg ccg cca ggt			1056
Glu Leu Asn Trp Thr Leu Pro Gly Gln Pro Asp Asn Thr Pro Pro Gly			
340	345	350	
ggt gaa ctg gtg ttt gaa cgc tgg cgt cgg cta agc gat aac agc cag			1104
Gly Glu Leu Val Phe Glu Arg Trp Arg Arg Leu Ser Asp Asn Ser Gln			
355	360	365	
tgg att cag gtt tcg ctg gtc ttc cag act tta cag cag atg cgt gat			1152
Trp Ile Gln Val Ser Leu Val Phe Gln Thr Leu Gln Gln Met Arg Asp			
370	375	380	
aaa acg ccg ctg tca tta aat acg ccg ccc gga gag gtg aaa ctg acc			1200
Lys Thr Pro Leu Ser Leu Asn Thr Pro Pro Gly Glu Val Lys Leu Thr			
385	390	395	400
ctg gca gga tgt gaa gag cga aat gcg cag ggc atg tgt tcg ttg gca			1248
Leu Ala Gly Cys Glu Glu Arg Asn Ala Gln Gly Met Cys Ser Leu Ala			
405	410	415	
ggt ttt acg caa atc gtg aat gaa gca cgc ata ccg gcg tgc agt ttg			1296
Gly Phe Thr Gln Ile Val Asn Glu Ala Arg Ile Pro Ala Cys Ser Leu			
420	425	430	
aga tct cat cac cat cac cat cac taa			1323
Arg Ser His His His His His His			
435	440		

<210> 2

<211> 440

<212> PRT

<213> Escherichia coli

<400> 2

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Val	Val	Ile	Val	Ser	Arg	His	Gly	Val	Arg	Ala	Pro	Thr	Lys	Ala	Thr	35	40	45	
Gln	Leu	Met	Gln	Asp	Val	Thr	Pro	Asp	Ala	Trp	Pro	Thr	Trp	Pro	Val	50	55	60	
Lys	Leu	Gly	Trp	Leu	Thr	Pro	Arg	Gly	Gly	Glu	Leu	Ile	Ala	Tyr	Leu	65	70	75	80
Gly	His	Tyr	Gln	Arg	Gln	Arg	Leu	Val	Ala	Asp	Gly	Leu	Leu	Ala	Lys	85	90	95	
Lys	Gly	Cys	Pro	Gln	Ser	Gly	Gln	Val	Ala	Ile	Ile	Ala	Asp	Val	Asp	100	105	110	
Glu	Arg	Thr	Arg	Lys	Thr	Gly	Glu	Ala	Phe	Ala	Ala	Gly	Leu	Ala	Pro	115	120	125	
Asp	Cys	Ala	Ile	Thr	Val	His	Thr	Gln	Ala	Asp	Thr	Ser	Ser	Pro	Asp	130	135	140	
Pro	Leu	Phe	Asn	Pro	Leu	Lys	Thr	Gly	Val	Cys	Gln	Leu	Asp	Asn	Ala	145	150	155	160
Asn	Val	Thr	Asp	Ala	Ile	Leu	Ser	Arg	Ala	Gly	Gly	Ser	Ile	Ala	Asp	165	170	175	
Phe	Thr	Gly	His	Arg	Gln	Thr	Ala	Phe	Arg	Glu	Leu	Glu	Arg	Val	Leu	180	185	190	
Asn	Phe	Pro	Gln	Ser	Asn	Leu	Cys	Leu	Lys	Arg	Glu	Lys	Gln	Asp	Glu	195	200	205	
Ser	Cys	Ser	Leu	Thr	Gln	Ala	Leu	Pro	Ser	Glu	Leu	Lys	Val	Ser	Ala	210	215	220	
Asp	Asn	Val	Ser	Leu	Thr	Gly	Ala	Val	Ser	Leu	Ala	Ser	Met	Leu	Thr	225	230	235	240
Glu	Ile	Phe	Leu	Leu	Gln	Gln	Ala	Gln	Gly	Met	Pro	Glu	Pro	Gly	Trp	245	250	255	
Gly	Arg	Ile	Thr	Asp	Ser	His	Gln	Trp	Asn	Thr	Leu	Leu	Ser	Leu	His	260	265	270	
Asn	Ala	Gln	Phe	Tyr	Leu	Leu	Gln	Arg	Thr	Pro	Glu	Val	Ala	Arg	Ser	275	280	285	
Arg	Ala	Thr	Pro	Leu	Leu	Asp	Leu	Ile	Met	Ala	Ala	Leu	Thr	Pro	His	290	295	300	
Pro	Pro	Gln	Lys	Gln	Ala	Tyr	Gly	Val	Thr	Leu	Pro	Thr	Ser	Val	Leu	305	310	315	320
Phe	Ile	Ala	Gly	His	Asp	Thr	Asn	Leu	Ala	Asn	Leu	Gly	Gly	Ala	Leu	325	330	335	
Glu	Leu	Asn	Trp	Thr	Leu	Pro	Gly	Gln	Pro	Asp	Asn	Thr	Pro	Pro	Gly	340	345	350	
Gly	Glu	Leu	Val	Phe	Glu	Arg	Trp	Arg	Arg	Leu	Ser	Asp	Asn	Ser	Gln	355	360	365	
Trp	Ile	Gln	Val	Ser	Leu	Val	Phe	Gln	Thr	Leu	Gln	Gln	Met	Arg	Asp	370	375	380	
Lys	Thr	Pro	Leu	Ser	Leu	Asn	Thr	Pro	Pro	Gly	Glu	Val	Lys	Leu	Thr	385	390	395	400
Leu	Ala	Gly	Cys	Glu	Glu	Arg	Asn	Ala	Gln	Gly	Met	Cys	Ser	Leu	Ala	405	410	415	
Gly	Phe	Thr	Gln	Ile	Val	Asn	Glu	Ala	Arg	Ile	Pro	Ala	Cys	Ser	Leu	420	425	430	
Arg	Ser	His	His	His	His	His	His									435	440		

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 <213> Artificial Sequence

<220>
 <223> primer

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<210> 4
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer

<400> 4
 gtttctggat ccttacaac tgcacgccg tat 33

<210> 5
 <211> 1901
 <212> DNA
 <213> Escherichia coli

<220>
 <221> misc_feature
 <222> 403
 <223> n = A,T,C or G

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 ctctccaccc ttgtgttggt atggctggac ccgctctga aaagttaacg aacgtaggcc 120
 tgatgcggcg cattagcatc gcatcaggca atcaataatg tcagatatga aaagcggaaa 180
 catatcgatg aaagcgatct taatcccatt tttatctctt ctgattccgt taaccccgca 240
 atctgcattc gctcagagtg agccggagct gaagctggaa agtgtggtga ttgtcagtcg 300
 tcatggtgtg cgtgctccaa ccaaggccac gcaactgatg caggatgtca cccagacgc 360
 atggccaacc tggccggtaa aactgggtga gctgacaccg cgnggtggtg agctaategc 420
 ctatctcgga cattaccaac gccagcgtct ggtagccgac ggattgctgg cgaaaaaggg 480
 ctgcccgcag tctggtcagg tcgcgattat tgctgatgtc gacgagcgta cccgtaaaac 540
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 taacgcgaac gtgactgacg cgatcctcag cagggcagga gggtaattg ctgactttac 720
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 cttgtgcctt aaacgtgaga aacaggacga aagctgttca ttaacgcagg cattaccatc 840
 ggaactcaag gtgagcgccg acaatgtctc attaaccggt gcggtaaagg tcgcatcaat 900
 gctgacggag atattttctc tgcaacaagc acagggaatg ccggagccgg ggtggggaag 960
 gatcaccgat tcacaccagt ggaacacctt gctaagtttg cataacgcgc aattttattt 1020
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ccacgaccgc	ggcatcactc	accgccagca	tcggcggcgt	atcgacaatc	accagatcgt	1860
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<210> 6

<211> 1901

<212> DNA

<213> Escherichia coli

<220>

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<223> n = A,T,C or G

<400> 6

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gctacaacgc	acgccagagg	ttgcccgcag	ccgcgccacc	ccgttattag	atttgatcaa	1080
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caactggacg	cttcccggtc	agccggataa	cacgcgcgca	ggtggtgaac	tggtgtttga	1260
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cctctttggc	ggtgttcaag	ccaaaacgcg	caaccagcag	gctggtgcc	acagaacgcc	1800
ccacgaccgc	ggcatcactc	accgccagca	tcggcggcgt	atcgacaatc	accagatcgt	1860
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<210> 7

<211> 1901

<212> DNA

<213> Escherichia coli

<220>
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 <222> (188)...(1483)

<221> misc_feature
 <222> 403
 <223> n = A,T,C or G

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tgatgcggcg cattagcatc gcatcaggca atcaataatg tcagatatga aaagcggaaa	180
catatcg atg aaa gcg atc tta atc cca ttt tta tct ctt ctg att ccg	229
Met Lys Ala Ile Leu Ile Pro Phe Leu Ser Leu Leu Ile Pro	
1 5 10	
tta acc ccg caa tct gca ttc gct cag agt gag ccg gag ctg aag ctg	277
Leu Thr Pro Gln Ser Ala Phe Ala Gln Ser Glu Pro Glu Leu Lys Leu	
15 20 25 30	
gaa agt gtg gtg att gtc agt cgt cat ggt gtg cgt gct cca acc aag	325
Glu Ser Val Val Ile Val Ser Arg His Gly Val Arg Ala Pro Thr Lys	
35 40 45	
gcc acg caa ctg atg cag gat gtc acc cca gac gca tgg cca acc tgg	373
Ala Thr Gln Leu Met Gln Asp Val Thr Pro Asp Ala Trp Pro Thr Trp	
50 55 60	
ccg gta aaa ctg ggt tgg ctg aca ccg cgn ggt ggt gag cta atc gcc	421
Pro Val Lys Leu Gly Trp Leu Thr Pro Arg Gly Gly Glu Leu Ile Ala	
65 70 75	
tat ctc gga cat tac caa cgc cag cgt ctg gta gcc gac gga ttg ctg	469
Tyr Leu Gly His Tyr Gln Arg Gln Arg Leu Val Ala Asp Gly Leu Leu	
80 85 90	
gcg aaa aag ggc tgc ccg cag tct ggt cag gtc gcg att att gct gat	517
Ala Lys Lys Gly Cys Pro Gln Ser Gly Gln Val Ala Ile Ile Ala Asp	
95 100 105 110	
gtc gac gag cgt acc cgt aaa aca ggc gaa gcc ttc gcc gcc ggg ctg	565
Val Asp Glu Arg Thr Arg Lys Thr Gly Glu Ala Phe Ala Ala Gly Leu	
115 120 125	
gca cct gac tgt gca ata acc gta cat acc cag gca gat acg tcc agt	613
Ala Pro Asp Cys Ala Ile Thr Val His Thr Gln Ala Asp Thr Ser Ser	
130 135 140	
ccc gat ccg tta ttt aat cct cta aaa act ggc gtt tgc caa ctg gat	661
Pro Asp Pro Leu Phe Asn Pro Leu Lys Thr Gly Val Cys Gln Leu Asp	
145 150 155	
aac gcg aac gtg act gac gcg atc ctc agc agg gca gga ggg tca att	709
Asn Ala Asn Val Thr Asp Ala Ile Leu Ser Arg Ala Gly Gly Ser Ile	
160 165 170	

gct gac ttt acc ggg cat cgg caa acg gcg ttt cgc gaa ctg gaa cgg Ala Asp Phe Thr Gly His Arg Gln Thr Ala Phe Arg Glu Leu Glu Arg 175 180 185 190	757
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gac gaa agc tgt tca tta acg cag gca tta cca tcg gaa ctc aag gtg Asp Glu Ser Cys Ser Leu Thr Gln Ala Leu Pro Ser Glu Leu Lys Val 210 215 220	853
agc gcc gac aat gtc tca tta acc ggt gcg gta agc ctc gca tca atg Ser Ala Asp Asn Val Ser Leu Thr Gln Gly Ala Val Ser Leu Ala Ser Met 225 230 235	901
ctg acg gag ata ttt ctc ctg caa caa gca cag gga atg ccg gag ccg Leu Thr Glu Ile Phe Leu Leu Gln Gln Ala Gln Gly Met Pro Glu Pro 240 245 250	949
ggg tgg gga agg atc acc gat tca cac cag tgg aac acc ttg cta agt Gly Trp Gly Arg Ile Thr Asp Ser His Gln Trp Asn Thr Leu Leu Ser 255 260 265 270	997
ttg cat aac gcg caa ttt tat ttg cta caa cgc acg cca gag gtt gcc Leu His Asn Ala Gln Phe Tyr Leu Leu Gln Arg Thr Pro Glu Val Ala 275 280 285	1045
cgc agc cgc gcc acc ccg tta tta gat ttg atc aag aca gcg ttg acg Arg Ser Arg Ala Thr Pro Leu Leu Asp Leu Ile Lys Thr Ala Leu Thr 290 295 300	1093
ccc cat cca ccg caa aaa cag gcg tat ggt gtg aca tta ccc act tca Pro His Pro Pro Gln Lys Gln Ala Tyr Gly Val Thr Leu Pro Thr Ser 305 310 315	1141
gtg ctg ttt atc gcc gga cac gat act aat ctg gca aat ctc ggc ggc Val Leu Phe Ile Ala Gly His Asp Thr Asn Leu Ala Asn Leu Gly Gly 320 325 330	1189
gca ctg gag ctc aac tgg acg ctt ccc ggt cag ccg gat aac acg ccg Ala Leu Glu Leu Asn Trp Thr Leu Pro Gly Gln Pro Asp Asn Thr Pro 335 340 345 350	1237
cca ggt ggt gaa ctg gtg ttt gaa cgc tgg cgt cgg cta agc gat aac Pro Gly Gly Glu Leu Val Phe Glu Arg Trp Arg Arg Leu Ser Asp Asn 355 360 365	1285
agc cag tgg att cag gtt tcg ctg gtc ttc cag act tta cag cag atg Ser Gln Trp Ile Gln Val Ser Leu Val Phe Gln Thr Leu Gln Gln Met 370 375 380	1333
cgt gat aaa acg ccg ctg tca tta aat acg ccg ccc gga gag gtg aaa Arg Asp Lys Thr Pro Leu Ser Leu Asn Thr Pro Pro Gly Glu Val Lys 385 390 395	1381
ctg acc ctg gca gga tgt gaa gag cga aat gcg cag ggc atg tgt tcg	1429

Leu Thr Leu Ala Gly Cys Glu Glu Arg Asn Ala Gln Gly Met Cys Ser
 400 405 410

ttg gca ggt ttt acg caa atc gtg aat gaa gca cgc ata ccg gcg tgc 1477
 Leu Ala Gly Phe Thr Gln Ile Val Asn Glu Ala Arg Ile Pro Ala Cys
 415 420 425 430

agt ttg taatgcataa aaaagagcat tcagttacct gaatgctctg aggctgatga 1533
 Ser Leu

caaacgaaga actgtctaata gcgtagaccg gaaaaggcgt tcacgccgca tccggccact 1593
 ttcagttttc ctctttctcg gagtaactat aaccgtaata gttatagccg taactgtaag 1653
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 Val Val Ile Val Ser Arg His Gly Val Arg Ala Pro Thr Lys Ala Thr
 35 40 45
 Gln Leu Met Gln Asp Val Thr Pro Asp Ala Trp Pro Thr Trp Pro Val
 50 55 60
 Lys Leu Gly Trp Leu Thr Pro Arg Gly Gly Glu Leu Ile Ala Tyr Leu
 65 70 75 80
 Gly His Tyr Gln Arg Gln Arg Leu Val Ala Asp Gly Leu Leu Ala Lys
 85 90 95
 Lys Gly Cys Pro Gln Ser Gly Gln Val Ala Ile Ile Ala Asp Val Asp
 100 105 110
 Glu Arg Thr Arg Lys Thr Gly Glu Ala Phe Ala Ala Gly Leu Ala Pro
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 Asp Cys Ala Ile Thr Val His Thr Gln Ala Asp Thr Ser Ser Pro Asp
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 Pro Leu Phe Asn Pro Leu Lys Thr Gly Val Cys Gln Leu Asp Asn Ala
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 Asn Val Thr Asp Ala Ile Leu Ser Arg Ala Gly Gly Ser Ile Ala Asp
 165 170 175
 Phe Thr Gly His Arg Gln Thr Ala Phe Arg Glu Leu Glu Arg Val Leu
 180 185 190
 Asn Phe Pro Gln Ser Asn Leu Cys Leu Lys Arg Glu Lys Gln Asp Glu
 195 200 205
 Ser Cys Ser Leu Thr Gln Ala Leu Pro Ser Glu Leu Lys Val Ser Ala
 210 215 220
 Asp Asn Val Ser Leu Thr Gly Ala Val Ser Leu Ala Ser Met Leu Thr
 225 230 235 240
 Glu Ile Phe Leu Leu Gln Gln Ala Gln Gly Met Pro Glu Pro Gly Trp
 245 250 255

Gly	Arg	Ile	Thr	Asp	Ser	His	Gln	Trp	Asn	Thr	Leu	Leu	Ser	Leu	His
			260					265					270		
Asn	Ala	Gln	Phe	Tyr	Leu	Leu	Gln	Arg	Thr	Pro	Glu	Val	Ala	Arg	Ser
		275					280					285			
Arg	Ala	Thr	Pro	Leu	Leu	Asp	Leu	Ile	Lys	Thr	Ala	Leu	Thr	Pro	His
		290				295					300				
Pro	Pro	Gln	Lys	Gln	Ala	Tyr	Gly	Val	Thr	Leu	Pro	Thr	Ser	Val	Leu
305					310					315					320
Phe	Ile	Ala	Gly	His	Asp	Thr	Asn	Leu	Ala	Asn	Leu	Gly	Gly	Ala	Leu
			325						330					335	
Glu	Leu	Asn	Trp	Thr	Leu	Pro	Gly	Gln	Pro	Asp	Asn	Thr	Pro	Pro	Gly
			340					345					350		
Gly	Glu	Leu	Val	Phe	Glu	Arg	Trp	Arg	Arg	Leu	Ser	Asp	Asn	Ser	Gln
		355					360					365			
Trp	Ile	Gln	Val	Ser	Leu	Val	Phe	Gln	Thr	Leu	Gln	Gln	Met	Arg	Asp
		370				375					380				
Lys	Thr	Pro	Leu	Ser	Leu	Asn	Thr	Pro	Pro	Gly	Glu	Val	Lys	Leu	Thr
385					390					395					400
Leu	Ala	Gly	Cys	Glu	Glu	Arg	Asn	Ala	Gln	Gly	Met	Cys	Ser	Leu	Ala
			405						410					415	
Gly	Phe	Thr	Gln	Ile	Val	Asn	Glu	Ala	Arg	Ile	Pro	Ala	Cys	Ser	Leu
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1				5					10					15		
ccg	caa	tct	gca	ttc	gct	cag	agt	gag	ccg	gag	ctg	aag	ctg	gaa	agt	96
Pro	Gln	Ser	Ala	Phe	Ala	Gln	Ser	Glu	Pro	Glu	Leu	Lys	Leu	Glu	Ser	
			20					25					30			
gtg	gtg	att	gtc	agt	cgt	cat	ggc	gtg	cgt	gct	cca	acc	aag	gcc	acg	144
Val	Val	Ile	Val	Ser	Arg	His	Gly	Val	Arg	Ala	Pro	Thr	Lys	Ala	Thr	
		35					40					45				
caa	ctg	atg	cag	gat	gtc	acc	cca	gac	gca	tgg	cca	acc	tgg	ccg	gta	192
Gln	Leu	Met	Gln	Asp	Val	Thr	Pro	Asp	Ala	Trp	Pro	Thr	Trp	Pro	Val	
	50					55					60					
aaa	ctg	ggc	gag	ctg	aca	ccg	cgc	ggc	ggc	gag	cta	atc	gcc	tat	ctc	240
Lys	Leu	Gly	Glu	Leu	Thr	Pro	Arg	Gly	Gly	Glu	Leu	Ile	Ala	Tyr	Leu	
65					70					75					80	
gga	cat	tac	tgg	cgt	cag	cgt	ctg	gta	gcc	gac	gga	tgg	ctg	cct	aaa	288
Gly	His	Tyr	Trp	Arg	Gln	Arg	Leu	Val	Ala	Asp	Gly	Leu	Leu	Pro	Lys	

85	90	95	
tgt ggc tgc ccg cag tct ggt cag gtc gcg att att gct gat gtc gac Cys Gly Cys Pro Gln Ser Gly Gln Val Ala Ile Ile Ala Asp Val Asp 100 105 110			336
gag cgt acc cgt aaa aca ggc gaa gcc ttc gcc gcc ggg ctg gca cct Glu Arg Thr Arg Lys Thr Gly Glu Ala Phe Ala Ala Gly Leu Ala Pro 115 120 125			384
gac tgt gca ata acc gta cat acc cag gca gat acg tcc agt ccc gat Asp Cys Ala Ile Thr Val His Thr Gln Ala Asp Thr Ser Ser Pro Asp 130 135 140			432
ccg tta ttt aat cct cta aaa act ggc gtt tgc caa ctg gat aac gcg Pro Leu Phe Asn Pro Leu Lys Thr Gly Val Cys Gln Leu Asp Asn Ala 145 150 155 160			480
aac gtg act gac gcg atc ctc gag agg gca gga ggg tca att gct gac Asn Val Thr Asp Ala Ile Leu Glu Arg Ala Gly Gly Ser Ile Ala Asp 165 170 175			528
ttt acc ggg cat tat caa acg gcg ttt cgc gaa ctg gaa cgg gtg ctt Phe Thr Gly His Tyr Gln Thr Ala Phe Arg Glu Leu Glu Arg Val Leu 180 185 190			576
aat ttt ccg caa tca aac ttg tgc ctt aaa cgt gag aaa cag gac gaa Asn Phe Pro Gln Ser Asn Leu Cys Leu Lys Arg Glu Lys Gln Asp Glu 195 200 205			624
agc tgt tca tta acg cag gca tta cca tcg gaa ctc aag gtg agc gcc Ser Cys Ser Leu Thr Gln Ala Leu Pro Ser Glu Leu Lys Val Ser Ala 210 215 220			672
gac tgt gtc tca tta acc ggt gcg gta agc ctc gca tca atg ctg acg Asp Cys Val Ser Leu Thr Gly Ala Val Ser Leu Ala Ser Met Leu Thr 225 230 235 240			720
gag ata ttt ctc ctg caa caa gca cag gga atg ccg gag ccg ggg tgg Glu Ile Phe Leu Leu Gln Gln Ala Gln Gly Met Pro Glu Pro Gly Trp 245 250 255			768
gga agg atc acc gat tca cac cag tgg aac acc ttg cta agt ttg cat Gly Arg Ile Thr Asp Ser His Gln Trp Asn Thr Leu Leu Ser Leu His 260 265 270			816
aac gcg caa ttt gat ttg cta caa cgc acg cca gag gtt gcc cgc agc Asn Ala Gln Phe Asp Leu Leu Gln Arg Thr Pro Glu Val Ala Arg Ser 275 280 285			864
cgc gcc acc ccg tta tta gat ttg atc aag aca gcg ttg acg ccc cat Arg Ala Thr Pro Leu Leu Asp Leu Ile Lys Thr Ala Leu Thr Pro His 290 295 300			912
cca ccg caa aaa cag gcg tat ggt gtg aca tta ccc act tca gtg ctg Pro Pro Gln Lys Gln Ala Tyr Gly Val Thr Leu Pro Thr Ser Val Leu 305 310 315 320			960

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ttt atc gcc gga cac gat act aat ctg gca aat ctc ggc ggc gca ctg      1008
Phe Ile Ala Gly His Asp Thr Asn Leu Ala Asn Leu Gly Gly Ala Leu
          325                      330                      335

gag ctc aac tgg acg ctt ccc ggt cag ccg gat aac acg ccg cca ggt      1056
Glu Leu Asn Trp Thr Leu Pro Gly Gln Pro Asp Asn Thr Pro Pro Gly
          340                      345                      350

ggt gaa ctg gtg ttt gaa cgc tgg cgt cgg cta agc gat aac agc cag      1104
Gly Glu Leu Val Phe Glu Arg Trp Arg Arg Leu Ser Asp Asn Ser Gln
          355                      360                      365

tgg att cag gtt tcg ctg gtc ttc cag act tta cag cag atg cgt gat      1152
Trp Ile Gln Val Ser Leu Val Phe Gln Thr Leu Gln Gln Met Arg Asp
          370                      375                      380

aaa acg ccg ctg tca tta aat acg ccg ccc gga gag gtg aaa ctg acc      1200
Lys Thr Pro Leu Ser Leu Asn Thr Pro Pro Gly Glu Val Lys Leu Thr
          385                      390                      395                      400

ctg gca gga tgt gaa gag cga aat gcg cag ggc atg tgt tcg ttg gca      1248
Leu Ala Gly Cys Glu Glu Arg Asn Ala Gln Gly Met Cys Ser Leu Ala
          405                      410                      415

ggt ttt acg caa atc gtg aat gaa gca cgc ata ccg gcg tgc agt ttg      1296
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 20          25          30
Val Val Ile Val Ser Arg His Gly Val Arg Ala Pro Thr Lys Ala Thr
 35          40          45
Gln Leu Met Gln Asp Val Thr Pro Asp Ala Trp Pro Thr Trp Pro Val
 50          55          60
Lys Leu Gly Glu Leu Thr Pro Arg Gly Gly Glu Leu Ile Ala Tyr Leu
 65          70          75          80
Gly His Tyr Trp Arg Gln Arg Leu Val Ala Asp Gly Leu Leu Pro Lys
 85          90          95
Cys Gly Cys Pro Gln Ser Gly Gln Val Ala Ile Ile Ala Asp Val Asp
100          105          110
Glu Arg Thr Arg Lys Thr Gly Glu Ala Phe Ala Ala Gly Leu Ala Pro
115          120          125
Asp Cys Ala Ile Thr Val His Thr Gln Ala Asp Thr Ser Ser Pro Asp

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130		135		140
Pro Leu Phe Asn Pro Leu Lys Thr Gly Val Cys Gln Leu Asp Asn Ala				
145		150		155
Asn Val Thr Asp Ala Ile Leu Glu Arg Ala Gly Gly Ser Ile Ala Asp				160
	165		170	175
Phe Thr Gly His Tyr Gln Thr Ala Phe Arg Glu Leu Glu Arg Val Leu				
	180		185	190
Asn Phe Pro Gln Ser Asn Leu Cys Leu Lys Arg Glu Lys Gln Asp Glu				
	195		200	205
Ser Cys Ser Leu Thr Gln Ala Leu Pro Ser Glu Leu Lys Val Ser Ala				
	210		215	220
Asp Cys Val Ser Leu Thr Gly Ala Val Ser Leu Ala Ser Met Leu Thr				
225		230		235
Glu Ile Phe Leu Leu Gln Gln Ala Gln Gly Met Pro Glu Pro Gly Trp				240
	245		250	255
Gly Arg Ile Thr Asp Ser His Gln Trp Asn Thr Leu Leu Ser Leu His				
	260		265	270
Asn Ala Gln Phe Asp Leu Leu Gln Arg Thr Pro Glu Val Ala Arg Ser				
	275		280	285
Arg Ala Thr Pro Leu Leu Asp Leu Ile Lys Thr Ala Leu Thr Pro His				
	290		295	300
Pro Pro Gln Lys Gln Ala Tyr Gly Val Thr Leu Pro Thr Ser Val Leu				
305		310		315
Phe Ile Ala Gly His Asp Thr Asn Leu Ala Asn Leu Gly Gly Ala Leu				
	325		330	335
Glu Leu Asn Trp Thr Leu Pro Gly Gln Pro Asp Asn Thr Pro Pro Gly				
	340		345	350
Gly Glu Leu Val Phe Glu Arg Trp Arg Arg Leu Ser Asp Asn Ser Gln				
	355		360	365
Trp Ile Gln Val Ser Leu Val Phe Gln Thr Leu Gln Gln Met Arg Asp				
	370		375	380
Lys Thr Pro Leu Ser Leu Asn Thr Pro Pro Gly Glu Val Lys Leu Thr				
385		390		395
Leu Ala Gly Cys Glu Glu Arg Asn Ala Gln Gly Met Cys Ser Leu Ala				
	405		410	415
Gly Phe Thr Gln Ile Val Asn Glu Ala Arg Ile Pro Ala Cys Ser Leu				
	420		425	430



FIGURE 1a

(SEQ ID NO:1-nucleotide sequence and SEQ ID NO:2-amino acid sequence)

Escherichia coli B Phytase Sequence

1
ATG AAA GCG ATC TTA ATC CCA TTT TTA TCT CTT CTG ATT CCG TTA ACC CCG
Met Lys Ala Ile Leu Ile Pro Phe Leu Ser Leu Leu Ile Pro Leu Thr Pro

CAA TCT GCA TTC GCT CAG AGT GAG CCG GAG CTG AAG CTG GAA AGT GTG GTG
Gln Ser Ala Phe Ala Gln Ser Glu Pro Glu Leu Lys Leu Glu Ser Val Val

ATT GTC AGT CGT CAT GGT GTG CGT GCT CCA ACC AAG GCC ACG CAA CTG ATG
Ile Val Ser Arg His Gly Val Arg Ala Pro Thr Lys Ala Thr Gln Leu Met

CAG GAT GTC ACC CCA GAC GCA TGG CCA ACC TGG CCG GTA AAA CTG GGT TGG
Gln Asp Val Thr Pro Asp Ala Trp Pro Thr Trp Pro Val Lys Leu Gly Trp

CTG ACA CCG CGN GGT GGT GAG CTA ATC GCC TAT CTC GGA CAT TAC CAA CGC
Leu Thr Pro Arg Gly Gly Glu Leu Ile Ala Tyr Leu Gly His Tyr Gln Arg

CAG CGT CTG GTA GCC GAC GGA TTG CTG GCG AAA AAG GGC TGC CCG CAG TCT
Gln Arg Leu Val Ala Asp Gly Leu Leu Ala Lys Lys Gly Cys Pro Gln Ser

GGT CAG GTC GCG ATT ATT GCT GAT GTC GAC GAG CGT ACC CGT AAA ACA GGC
Gly Gln Val Ala Ile Ile Ala Asp Val Asp Glu Arg Thr Arg Lys Thr Gly

GAA GCC TTC GCC GCC GGG CTG GCA CCT GAC TGT GCA ATA ACC GTA CAT ACC
Glu Ala Phe Ala Ala Gly Leu Ala Pro Asp Cys Ala Ile Thr Val His Thr

CAG GCA GAT ACG TCC AGT CCC GAT CCG TTA TTT AAT CCT CTA AAA ACT GGC
Gln Ala Asp Thr Ser Ser Pro Asp Pro Leu Phe Asn Pro Leu Lys Thr Gly

GTT TGC CAA CTG GAT AAC GCG AAC GTG ACT GAC GCG ATC CTC AGC AGG GCA
Val Cys Gln Leu Asp Asn Ala Asn Val Thr Asp Ala Ile Leu Ser Arg Ala

GGA GGG TCA ATT GCT GAC TTT ACC GGG CAT CGG CAA ACG GCG TTT CGC GAA
Gly Gly Ser Ile Ala Asp Phe Thr Gly His Arg Gln Thr Ala Phe Arg Glu

CTG GAA CGG GTG CTT AAT TTT CCG CAA TCA AAC TTG TGC CTT AAA CGT GAG
Leu Glu Arg Val Leu Asn Phe Pro Gln Ser Asn Leu Cys Leu Lys Arg Glu

AAA CAG GAC GAA AGC TGT TCA TTA ACG CAG GCA TTA CCA TCG GAA CTC AAG
Lys Gln Asp Glu Ser Cys Ser Leu Thr Gln Ala Leu Pro Ser Glu Leu Lys

GTG AGC GCC GAC AAT GTC TCA TTA ACC GGT GCG GTA AGC CTC GCA TCA ATG
Val Ser Ala Asp Asn Val Ser Leu Thr Gly Ala Val Ser Leu Ala Ser Met

CTG ACG GAG ATA TTT CTC CTG CAA CAA GCA CAG GGA ATG CCG GAG CCG GGG
Leu Thr Glu Ile Phe Leu Leu Gln Gln Ala Gln Gly Met Pro Glu Pro Gly

TGG GGA AGG ATC ACC GAT TCA CAC CAG TGG AAC ACC TTG CTA AGT TTG CAT
Trp Gly Arg Ile Thr Asp Ser His Gln Trp Asn Thr Leu Leu Ser Leu His

AAC GCG CAA TTT TAT TTG CTA CAA CGC ACG CCA GAG GTT GCC CGC AGC CGC
Asn Ala Gln Phe Tyr Leu Leu Gln Arg Thr Pro Glu Val Ala Arg Ser Arg

GCC ACC CCG TTA TTG GAT TTG ATC ATG GCA GCG TTG ACG CCC CAT CCA CCG
Ala Thr Pro Leu Leu Asp Leu Ile Met Ala Ala Leu Thr Pro His Pro Pro



FIGURE 1b

CAA AAA CAG GCG TAT GGT GTG ACA TTA CCC ACT TCA GTA CTG TTT ATT GCC
Gln Lys Gln Ala Tyr Gly Val Thr Leu Pro Thr Ser Val Leu Phe Ile Ala

GGA CAC GAT ACT AAT CTG GCA AAT CTC GGC GGC GCA CTG GAG CTC AAC TGG
Gly His Asp Thr Asn Leu Ala Asn Leu Gly Gly Ala Leu Glu Leu Asn Trp

ACG CTT CCC GGT CAG CCG GAT AAC ACG CCG CCA GGT GGT GAA CTG GTG TTT
Thr Leu Pro Gly Gln Pro Asp Asn Thr Pro Pro Gly Gly Glu Leu Val Phe

GAA CGC TGG CGT CGG CTA AGC GAT AAC AGC CAG TGG ATT CAG GTT TCG CTG
Glu Arg Trp Arg Arg Leu Ser Asp Asn Ser Gln Trp Ile Gln Val Ser Leu

GTC TTC CAG ACT TTA CAG CAG ATG CGT GAT AAA ACG CCG CTG TCA TTA AAT
Val Phe Gln Thr Leu Gln Gln Met Arg Asp Lys Thr Pro Leu Ser Leu Asn

ACG CCG CCC GGA GAG GTG AAA CTG ACC CTG GCA GGA TGT GAA GAG CGA AAT
Thr Pro Pro Gly Glu Val Lys Leu Thr Leu Ala Gly Cys Glu Glu Arg Asn

GCG CAG GGC ATG TGT TCG TTG GCA GGT TTT ACG CAA ATC GTG AAT GAA GCA
Ala Gln Gly Met Cys Ser Leu Ala Gly Phe Thr Gln Ile Val Asn Glu Ala

CGC ATA CCG GCG TGC AGT TTG AGA TCT CAT CAC CAT CAC CAT CAC TAA 1323
Arg Ile Pro Ala Cys Ser Leu Arg Ser His His His His His His End



Amino acid sequence for E. coli appA (wild type) (SEQ ID NO:8)

MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVTP
DAWPTWPVKLGWLTPRGGELIAYLGHYQRQRLVADGLLAKKGCPQSGQVAIIA
DVDERTRKTEGAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDNA
NVTDAILESRAGGSIAFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCSLTQA
LPSELKVSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRTDSHQWNTLL
SLHNAQFYLLQRTPEVARSRATPLLDLIKALTTPHPQKQAYGVTLPTS SVLFIAGH
DTNLANLGGALELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSLVFQTL
QQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVNEARIPACSL

Bold-Underlined amino acid residues are shown below in the modified appA enzyme
(SEQ ID NO:10)

MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVTP
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ADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDNA
NVTDAILEEAGGSIAFTGHYQTAFRELERVLNFPQSNLCLKREKQDESCSLTQAL
PSELKVSADCVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRTDSHQWNTLLS
LHNAQFDLLQRTPEVARSRATPLLDLIKALTTPHPQKQAYGVTLPTS SVLFIAGH
DTNLANLGGALELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSLVFQTL
QQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVNEARIPACSLRS
~~HL~~

Figure 8